



Evaluation of CDX2 Expression in Gastric Precancerous Lesions and Gastric Carcinoma

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ABSTRACT

Introduction: Gastric carcinoma is the fifth most common and fourth fatal cancer in the world. The current TNM staging system together with clinicopathological characteristics are not sufficient to detect prognosis. Expression of CDX2 in gastric mucosa is strongly associated with precancerous lesions and cancer.

Aim/Objectives: The aim of the study was to evaluate CDX2 expression in different gastric premalignant lesions including intestinal metaplasia, chronic atrophic gastritis, adenoma / dysplasia and in gastric carcinoma and to evaluate the expression between histopathological types, grade and pTNM stages in gastric carcinoma.

Methods: It was a cross sectional observational study conducted at Sir Salimullah Medical College & Mitford Hospital, Dhaka over a period of 2 years. CDX2 expression was examined by immunohistochemical method in 77 formalin fixed-paraffin-embedded surgical specimens of gastric precancerous lesions and gastric cancer. The association between CDX2 expression and gastric precancerous lesion was evaluated. The association between CDX2 expression and gastric cancer including histopathological types, grade and pT and pN stages were evaluated. Association between CDX2 expression and specimen type was also evaluated.

Results: The mean age of the patient was 55.6±11.9 years ranging from 33 to 86 years and majority of the cases were male (n=53, 68.8%). Among total 77 cases, there were 60 malignant and 17 precancerous lesions. Majority of the cases were endoscopic biopsy specimens (n=42, 54.5%). CDX2 expression was significantly high in precancerous lesions (p=0.039). Among malignant tumors, significantly high expression was observed in well and moderately differentiated (p=0.001) and in low-grade malignant tumors (p<0.001) and in biopsied specimens (p<0.001), no significant association was observed with pT and pN stages. Significantly high expression was associated with intestinal type (p=0.001) and tubular adenocarcinoma (p=0.001) cases.

Conclusion: CDX2 is significantly associated with precancerous lesion and intestinal type of low-grade GC. This marker can predict the behavior and prognosis of GC and plan suitable line of treatment and could be potential for novel therapeutic targets.

Key Words: Evaluation, Gastric Carcinoma, Precancerous Lesions, CDX2 Expression, pTNM stages

INTRODUCTION

Gastric cancer (GC) is the fifth most commonly diagnosed and seventh most prevalent cancer.¹ Over one million cases of gastric cancer are diagnosed each year around the world. About 75.3% cases of gastric carcinoma occur in Asia.² However, the mortality rate is also the highest in Asia consisting of about 74.8%.³ Gastric carcinogenesis is a multifactorial and multi-step process, characterized by a complex interplay between genetic and environmental factors. Regarding environmental factors, chronic infection with *Helicobacter pylori* plays a central role.⁴ Amidst diverse

risk factors smoking, alcohol usage, ingestion of smoked and salted food, pernicious anemia, previous gastric operations, *Peutz-Jeghers* syndrome, *Li-Fraumeni* syndrome and hereditary diffuse gastric cancer syndrome are the most important causes.⁵ Precursor lesions of the stomach as well as cancer develop by gradual accumulation of mutations, usually initiated by an inflammatory process triggered by *H. pylori*, which may progress to multifocal atrophic gastritis, intestinal metaplasia (IM), chronic atrophic gastritis, dysplastic changes in mucosa and ultimately adenocarcinoma.⁶ Among the premalignant lesions, IM is further classified into complete and incomplete type according to the types

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of cells and mucin secretion status. Incomplete intestinal metaplasia poses greater risk of gastric carcinoma and is considered as precursor lesion for gastric carcinogenesis.⁷ Familial Adenomatous Polyposis (FAP) is the most common form of heritable gastric cancer; nevertheless, lifetime risk of development of gastric cancer is only 1% indicating several other environmental factors might play a role to transform these polyps into adenocarcinoma.⁸ The most convenient classification for gastric cancer is the Lauren Classification. It categorizes GC into two main histological categories: intestinal and diffuse type.⁹ Diffuse type GC infiltrates deeply into the stomach without forming obvious mass lesions but spread widely in the gastric wall.¹⁰ According to the World Health Organization (WHO) classification, the vast majority of gastric cancers are adenocarcinomas, divided into papillary, tubular, mucinous (colloid), and poorly cohesive carcinomas (including signet-ring cell carcinoma and other variants).¹¹ Gastric carcinoma has several prognostic and predictive factors. Histopathological staging is the most important prognostic indicator. Other well-known indicators include Japanese ethnicity, female gender, older age and tumor confined to distal stomach. In besides, nuclear grade, lympho-vascular invasion and perineural invasion are also regarded as significant prognostic factors. These factors are widely used to determine application of neoadjuvant therapy in gastric cancer.¹² CDX2 is a homeodomain protein expressed by caudal type homeobox transcription factor. It is a potentially valuable biomarker that can be implemented in routine clinical diagnostic setting of gastric cancer.¹³ CDX2 binds to CDX1 promoter region in the intestinal metaplasia and up regulates the transcriptional activity of CDX1 gene in human gastric cancer.¹⁴ CDX2 has a tumor suppressive role in the pathogenesis and progression of gastric carcinoma. It has been established that *H. pylori* induced inflammatory pathway causes activation of IL-6/STAT-3 signaling pathway which suppress CDX2 expression and leads to gastric cancer.¹⁵ Promoter methylation often leads to loss of function of this specific transcription factor CDX2 in primary gastric cancer.¹⁶ The ectopic expression of CDX2 plays a vital role in intestinal metaplasia genesis as well as intestinal type of gastric adenocarcinoma. Positive expression of this marker is significantly associated with male gender, well and moderately differentiated GC, and inversely correlated with stage. High rate of 5-years survival was noted in CDX2 positive gastric cancer cases.¹⁷ Establishment of supreme personalized medication and alleviation of multidrug resistance (MDR) are two major strategies regarding gastric oncotherapy. Implication of DNA Methyl Transferase-1 (DNMT1) inhibitors can salvage the expression of CDX2 in this regard.¹⁸ Furthermore, down regulation of CDX2 using RANi, might reverse the progression of MDR in gastric cancer both in vitro and in vivo.¹⁹

MATERIALS & METHODS

Study Design: This was a cross sectional observational study.

Place of Study: The study was carried out at the Department of Pathology; Sir Salimullah Medical College (SSMC), Dhaka and immune histochemical study was carried out at Square Hospital Ltd. Dhaka, Bangladesh.

Study Period: This study was carried out from September 2019 to August 2021.

Study Population: Patient who were histopathologically diagnosed as gastric cancer and precancerous lesion in pathology department, SSMC and other institutes of Dhaka city.

Study Sample: Specimen received at the Department of Pathology of SSMC, Dhaka from different departments of SSMC and other institutes of Dhaka city.

Sample Size: Calculated sample size was 82. However, for convenience and time constraint, this study was conducted by taking 77 samples that fulfilled the inclusion and exclusion criteria.

Inclusion Criteria

- Patients histopathologically diagnosed as a case of primary gastric carcinoma and precancerous lesion.

Exclusion Criteria

- Patients previously treated with chemotherapy or radiotherapy for gastric carcinoma.
- Patients with other tumors like lymphoma, carcinoid tumor and malignant GIST.
- Patients histopathologically diagnosed as a case of metastatic tumor.

Sample Collection and Processing: In this study, a total of 77 formalin fixed paraffin embedded (FFPE) blocks of GC and precancerous lesions were selected for histopathological examination. Sections were studied under light microscope and were histologically classified, graded and staged according to WHO Classification 2019, Lauren Classification and pTNM staging system.

Methods of Data Collection: Data was recorded on variables of interest by interviewing and using the structured questionnaire after taking properly informed written consent from the patients. Patients were eligible for inclusion if they were histopathologically diagnosed as gastric carcinoma and precursor lesion fulfilling specified criteria. Surgically resected specimens and endoscopic biopsy samples from stomach were received in 10% neutral buffered formalin. Gross features of received specimens were recorded. Representative part of tissue was taken and routine processing was done to prepare hematoxylin and eosin-stained slides. Diagnosis of gastric cancer and precursor lesions was done by histo-

pathological evaluation during specified time duration. The cases were subdivided according to Lauren classification and WHO classification system, 2019. The prognostic parameters including histologic type, tumor grading, depth of invasion, and regional lymph nodes metastases were assessed.

Evaluation of CDX2 Expression: The IHC scoring system was based on intensity of reactivity and the percentage of reactive cells. Nuclear staining was considered as immunopositive.²⁰ The scoring was done by using the 40X objective lens and counting at least 100 cells for immunoreactivity in 10 fields. The final immunoreactive score was determined by multiplying the intensity and extent of positivity scores of stained cells. The minimum score was 0 and the maximum score was 12. Score of <3 was considered as the negative expression group, and those with a score of ≥ 3 was considered as the positive expression group.

Scoring for CDX2 protein (Chai et al., 2021):²⁰ The result of CDX2 protein immunohistochemistry was quantified as scores, according to the following method.

Intensity score(I)	Staining pattern
0	Negative (no staining)
1	Weakly positive
2	Moderately positive
3	Strongly positive
Proportion score(P)	Percentage of stained cells
0	<5% stained cells
1	>5% to 25% stained cells
2	>25% to 50% stained cells
3	>50% to 75% stained cells
4	>75% stained cells
Final score (IxP)	Pattern of expression of CDX2
> 3	Positive
<3	Negative

Statistical Analysis: The data collected were entered into Excel worksheet to generate a master data sheet. The statistical analysis was conducted using SPSS (Statistical Package for the Social Science) version 26 software. The findings of the study were presented by frequency, and percentage in tables. Means, median and standard deviations for continuous variables and frequency distributions for categorical variables were used to describe the characteristics of the total sample. Chi-square test, Fisher Exact test and Mann Whitney U Test assessed association between categorical variables. The $p < 0.05$ was considered as significant. Here, all p values were two sided.

RESULTS

Total 77 cases diagnosed as gastric precancerous lesions and gastric carcinoma were included in the study. Expression of

CDX2 was evaluated in 77 formalin fixed and paraffin embedded (FFPE) blocks of GC and precancerous lesions by immunohistochemistry. Cases were classified according to Lauren,⁹ WHO (2019) classification and pTNM staging as per AJCC respectively. Results were recorded and analyzed accordingly. Following observations were formulated.

Table 1: Demographic characteristics of patients (n=77)

Variables	Frequency(n)	Percentage (%)
Age		
Up to 40	10	13.0
41-50	19	24.7
51-60	26	33.7
61-70	17	22.1
>70	5	6.5
Total	77	100.0
Mean + SD, Range (min-max)	55.6 $\pm$ 11.9, 33.0-86.0	
Gender		
Male	53	68.8
Female	24	31.2
Smoking habit		
Smoker	42	54.5
Non-smoker	35	45.5
Food habit		
Normal diet	16	20.7
Increased salt intake	40	51.9
Less vegetable intake	25	32.4
Increased red meat intake	13	16.8
Location of lesion, malignant lesion		
Antrum	20	33.3
Pylorus	18	30.0
Body	13	21.7
Cardia	4	6.7
Body and pylorus	4	6.7
Body and antrum	1	1.6
Location of lesion, precancerous lesion		
Antrum	8	47.1
Pylorus	2	11.7
Body	7	41.2
Cardia	0	0.0
Body and pylorus	0	0.0
Body and antrum	0	0.0

Table 1 shows that, among the 77 patients, 19 (24.7%) were in 41-50 years age group and 26 (33.7%) were in 51-60 years age group. The mean ($\pm$ SD) age of the patients was 55.6 $\pm$ 11.9 years where minimum age was 33 years and maximum age was 86 years. Respondent's age was categorized into 10 class intervals. Among the 77 patients, 53 patients (68.8%) were male and the rest 24 (31.2%) were female. Among the 77 patients, 42 (54.5%) were smokers while 35 (45.5%) were non-smokers. Regarding dietary habit, among

the 77 patients, 16 (20.7%) had normal diet while increased salt intake was observed among 40 (51.9%) patients. Again, 25 (32.4%) patients had less vegetable in diet. Increased red meat consumption was observed among 13 (16.8%) patients. Among the 60 cases with malignant lesion, 20 (33.3%) cases had lesion in the antrum, 18 (30.0%) cases had in the pylorus and 13 (21.7%) cases had in the body. Again, among the 17 cases with precancerous lesion, 8 (47.1%) cases had lesion in the antrum, 2 (11.8%) cases had lesion in the pylorus and 7 (41.2%) cases had lesion in the body.

Table 2: Distribution of cases by type of specimen (n=77)

Type of specimen	Frequency (n)	Percentage (%)
Endoscopic biopsy	42	54.5
Partial gastrectomy	34	44.2
Total gastrectomy	1	1.3
Total	77	100.0

Table 2 shows that, among the 77 specimens, majority 42 (54.5%) were endoscopic biopsy specimens, while 34 (44.2%) were partial gastrectomy specimens. Only one case was total gastrectomy specimen (1.3%).

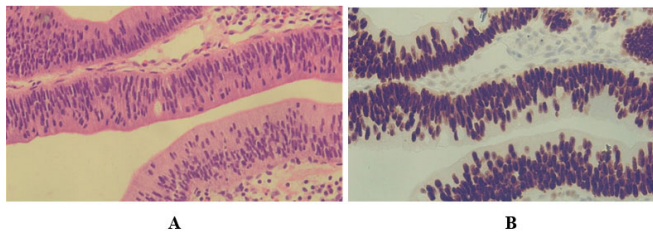


Figure 1: Photomicrograph showing high-grade adenoma/dysplasia (A. H & E stain, x 400 and B. corresponding CDX2 immunostain) in endoscopic biopsy sample. It shows positive CDX2 expression (CDX2 score =12).

Table 3: Distribution of cases by type of lesion according to WHO classification (n=77).

Type of lesion	Frequency (n)	Percentage (%)
Malignant lesion (n=60)		
Tubular adenocarcinoma	40	66.7
Poorly cohesive carcinoma and other cell type	11	18.3
Poorly cohesive carcinoma including signet ring cell type	2	3.3
Tubular (solid) adenocarcinoma	5	8.4
Mucinous adenocarcinoma	2	3.3
Precursor lesion (n=17)		
Adenoma /dysplasia	8	47.1
Chronic gastritis with intestinal metaplasia	8	47.1
Chronic atrophic gastritis	1	5.8

Table 3 shows that, majority of the cases 60 (77.9%) were malignant lesions (adenocarcinoma) and 17(22.1%) were precursor lesions. Among the 60 malignant cases, 40 (66.7%) were tubular adenocarcinoma, 11(18.3%) were poorly cohesive carcinoma and other cell types, 2(3.3%) were poorly cohesive carcinoma including signet ring cell type, 5(8.4%) cases were tubular (solid) adenocarcinoma and 2 (3.3%) were mucinous adenocarcinoma. Among the 17 precancerous lesions, 8 (47.1%) cases were adenoma/dysplasia, 8 (47.1%) were chronic gastritis with intestinal metaplasia and 1 (5.8%) case was chronic atrophic gastritis.

Table 4: Distribution of precancerous cases (adenoma/dysplasia) by grading (WHO, 2019) (n=8)

Adenoma/ dys-plasia	Frequency (n)	Percentage (%)
Low	3	37.5
High	5	62.5
Total	8	100.0

Table 4 shows that, among the 8-adenoma / dysplasia cases, 3 (37.5%) cases were low grade, while 5 (62.5%) cases were high grade. Here, among the 17 precancerous lesions, only adenoma/ dysplasia group was graded according to WHO 2019 classification. For classification of precancerous group, chronic gastritis with intestinal metaplasia (n=8), immunohistochemical stains for gastric mucin (EMA, MUC5AC and MUC6) and intestinal mucin (MUC2) are required which were not included in this study (Rugge, 2019). In addition, there was only one case of chronic atrophic gastritis, so it could not be categorized.

Table 5: Distribution of malignant cases by two-tier grading system (WHO, 2019) (n=60)

Two-tier grading system	Frequency (n)	Percentage (%)
Low	40	66.7
High	20	33.3
Total	60	100.0

Table 5 shows, among the 60 malignant cases, 40 (66.7%) cases were low grade and 20 (33.3%) cases were high grade.

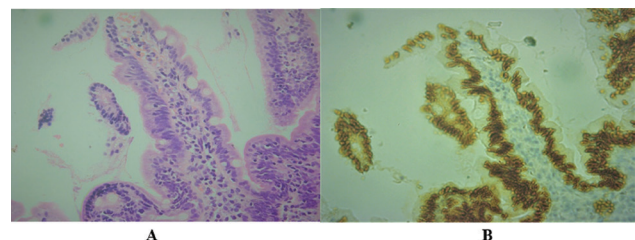


Figure 2: Photomicrograph showing chronic gastritis with intestinal metaplasia (A. H & E stain x 400 and B. corresponding CDX2 immunostain x 400) in endoscopic biopsy sample. It shows positive CDX2 expression (CDX2 score =12).

Table 6: Association of cases by CDX2 expression and histopathological grading among malignant lesion (n=60)

Histopathological grading	Negative	Positive	Total	p Value
Well differentiated	2(15.4%)	11(84.6%)	13(100.0%)	0.001 ^s
Moderately differentiated	7(25.9%)	20(74.1%)	27(100.0%)	
Poorly differentiated	14(70.0%)	6(30.0%)	20(100.0%)	

Table 6 shows that, among the 13 cases with well differentiated malignant lesion, 11 (84.6%) had positive expression. Out of the 27 cases with moderately differentiated malignant lesion, 20 (74.1%) had positive expression while among the 20 cases with poorly differentiated malignant lesion, 6 (30.0%) had positive expression. Fisher Exact test shows that there was significant statistical difference of CDX2 expression among the groups regarding histopathological grading of malignant lesion ($p=0.001$).

Table 7: Association of CDX2 expression with type of lesion (n=77)

Type of lesion	Negative	Positive	Total	p Value
Malignant lesion	23(38.3%)	37(61.7%)	60(100.0%)	0.039 ^s
Precursor lesion	2(11.8%)	15(88.2%)	17(100.0%)	

Table 7 shows that, among the 60 cases with malignant lesion, 37 (61.7%) had positive CDX2 expression while among the 17 cases with precursor lesion, 15 (88.2%) cases had positive CDX2 expression. Chi square test shows that there was significant statistical difference of CDX2 expression between the groups regarding type of lesion ($p=0.039$).

Table 8: Association of CDX2 expression and histopathological type of gastric carcinoma by Lauren classification (n=60)

Type of cancer	Negative	Positive	Total	p Value
Intestinal	10(23.8%)	32(76.2%)	42(100.0%)	0.001 ^s
Diffuse	10(76.9%)	3(23.1%)	13(100.0%)	
Indeterminate	3(60.0%)	2(40.0%)	5(100.0%)	

Table 8 shows the association of CDX2 expression and histopathological type of gastric carcinoma in both biopsy and resected specimens. It shows that among the 42 cases with intestinal type gastric cancer, 32 (76.2%) had positive expression. Out of the 13 cases with diffuse type gastric carcinoma, 3 (23.1%) had positive expression while among the 5 cases with indeterminate type gastric carcinoma, 2 (40.0%)

had positive expression. Fisher Exact test shows that there was significant statistical difference of CDX2 expression among the groups regarding histopathological type of gastric carcinoma by Lauren classification ($p=0.001$).

Table 9: Association of cases by CDX2 expression and pT staging (n=35)

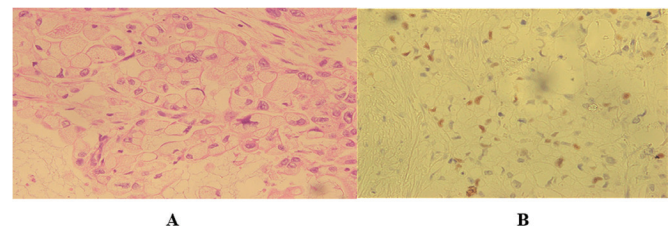
pT staging	Negative	Positive	Total	p Value
pT 2	1(50.0%)	1(50.0%)	2(100.0%)	0.321 ^{ns}
pT3	14(53.8%)	12(46.2%)	26(100.0%)	
pT4	6(85.7%)	1(14.3%)	7(100.0%)	

Table 9 shows that, between the two cases with pT2 staging, 1 (50.0%) had positive expression and out of the 26 cases with pT3 staging, 12 (46.2%) had positive expression. Among the 7 cases with pT4 staging, only 1 (14.3%) had positive expression. Fisher Exact test shows that there was no significant statistical difference of CDX2 expression among the groups regarding pT staging ($p=0.321$).

Table 10: Association between CDX2 expression and type of specimen in malignant cases (n=60)

Type of specimen	Negative	Positive	Total	p value
Resected specimen	21 (91.3%)	14 (37.8%)	35 (58.3%)	<0.001 ^s
Biopsy specimen	2 (8.7%)	23 (62.2%)	25 (41.7%)	

Table 10 shows that regarding 60 malignant cases, in 35 cases with resected specimens, 14 (37.8%) had positive expression, while among the 25 cases with endoscopic biopsy specimens, 23 (62.2%) had positive expression. Chi-square test shows that there was significant statistical difference of CDX2 expression between the groups regarding specimen type ($p<0.001$).

**Figure 3:** Photomicrograph showing diffuse type gastric cancer, poorly cohesive carcinoma including signet ring cell type, high grade (A. H & E stain x 400 and B. corresponding CDX2 immunostain x 400) in endoscopic biopsy specimen. It shows negative CDX2 expression (CDX2 score =1).

DISCUSSION

The present cross sectional observational study was conducted among 77 cases of gastric cancer precancerous lesions. This study was done to evaluate CDX2 expression among gastric cancer and precancerous lesions and to determine association of CDX2 expression with histopathological type, grade, and stage. The mean age of the patients was 55.6±11.9 years, one third of the patients were from 51-60 years age group. A study was conducted to determine the clinical and pathological profile of carcinoma stomach in Bangladesh where the mean age of the patients was found to be 51.05±14.98 years.¹⁹ However, the mean age of the Iranian patients was 60.58±11.88 year,^{20,21} while in Korea, it was 63 years.²² The peak age incidence of gastric cancer of South Asian countries was found to be in the fifth decade of life, which is about a decade or two earlier compared to the findings in developed countries. It is possible that the earlier age occurrence of gastric cancer is related to the life expectancy in the country, rather than any special demographic feature of gastric carcinoma.²³ Gastric cancer is more prevalent among male gender. In developed countries, gastric cancer is 2.2 times more common among male gender. In developing countries, this ratio is 1.83.¹ Majority of the patients (n=53, 68.8%) of this study were male which is consistent with studies by Islam et al.,²⁴ and Halder et al.²⁵ A possible explanation might be the protective effect of estrogen which may lower the risk of gastric cancer in women. Several factors like, differences in diet and occupational exposure may contribute to increased gastric cancer incidence in male.²⁶ In this study among the 77 patients, majority of the cases (n=42, 54.5%) were smokers. This result is similar to the study by Halder et al.²⁵ In the current study among the 77 patients, 16 (20.7%) had normal dietary habit. Increased consumption of salt can potentiate the carcinogenic effects of N-methyl-N-nitro-N-nitrosoguanidine (MNNG). Salt is known to erode the mucosal barrier of the stomach, thereby leading to inflammation.¹ High salt intake was observed among majority (n=40, 51.9%) patients of the current study. However, Bonequi et al.²⁷ observed, fruit and vegetable consumption were associated with a moderately decreased risk of GC. ²⁷ Near about one third of the patients (n=25, 32.4%) of the present study had less vegetable in diet. Increased red meat consumption was observed among 13 (16.8%) patients. In this study, antrum was the most common site for both gastric carcinoma (n=20, 33.3%) and precancerous cases (n=8, 47.1%). A study including combined group of gastric dysplasia and cancer showed that, IM in the antrum was significantly higher in the dysplasia group compared to the control group (p=0.016). The IM in the body was significantly higher in the dysplasia and cancer group compared to the control group (p<0.001) and p <0.001, respectively).^{22, 23} However, Jung et al.²⁸ proposed that majority of adenoma cases occurred in the antrum. Nevertheless, Yaprak et al.⁵ showed

that carcinoma in the antrum and pylorus were more frequent site (43%) than in corpus (29%). In the present study majority of the cases presented with ulcer proliferative type of lesion (n=28, 36.4 %). Qurieshi et al.²⁹ observed similar result where majority (n=29, 35.8%) cases had ulcer proliferative type of lesion. Nevertheless, Bhattarai et al.³⁰ showed that majority of the cases were ulcerative lesion (n=30, 46.9%). In this study, the majority (n=42, 54.5%) of the cases were endoscopic biopsy specimens. This is comparable to the study by Qin et al.,³¹ and Shin et al.,³² and where majority of the cases were endoscopic biopsy specimens. In contrast, Yaprak et al.,⁵ analyzed majority of cases from resected specimens. According to this study majority of the cases were malignant lesion (n=60, 77.9%) and the rest were precancerous lesion (n=17, 22.1%). A study conducted by Talukder et al.,³³ found gastric carcinoma in 67.23% of cases. However, the study by Islam et al.,²⁴ conducted at Delta Hospital Ltd Dhaka, showed 639(41.35%) malignant tumors, 493 (32.05%) chronic active gastritis and 150 (9.75%) with chronic gastritis with IM. Discordance among the results may be due to relatively small sample size in this study. In the current study, among the 60 malignant cases, tubular adenocarcinoma was the most common type (n=40, 66.7%), followed by poorly cohesive carcinomas and other cell types (n=11, 18.3%). This result corresponds to the study by Daun et al.,³⁴ where tubular adenocarcinoma was 69.6% cases and poorly cohesive carcinoma was 14.8% cases. In the present study among the 17 precancerous cases, 8 (47.1%) cases were adenoma/dysplasia, 8 (47.1%) cases were chronic gastritis with intestinal metaplasia and only 1(5.8%) case was chronic atrophic gastritis which is similar to the study conducted by Sandhya et al.³⁵ consisting of 50% chronic gastritis with IM and 4% chronic atrophic gastritis cases. In this study, according to Lauren classification, majority (n=42, 70%) of the cases were intestinal-type gastric cancer while 13 (21.7%) were diffuse type of carcinoma and 5 cases (8.3%) were indeterminate type cancer. Sandhya et al.,³⁵ reported 34 (68%) cases of intestinal type carcinoma and 9 (18%) cases of diffuse type carcinoma with 6 (12%) cases of mixed type carcinoma, which is similar to the present study. This study revealed, majority (n=27, 45%) of the GC cases were moderately differentiated type which is similar to the study of Halder et al.²⁵ In the present study, among the 35 cases from resected specimens, majority (n=26, 74.3%) of cases tumor invaded the subserosa pT3. However, pN3 staging were observed in majority 13 (37.2%) of the cases while none of the cases of the current study had distant metastasis (M0) as no sample was available for M staging. This is similar to studies by Daun et al.³⁴ and Zilberstein et al.,³⁸ where majority of the cases belonged to pT3 stages. Nevertheless, Yaprak et al.⁵ showed majority of cases belonged to pN3 stages, which is relevant to this study. In the current study among 60 malignant cases 35 cases with resected specimens, 14 (37.8%) had positive expression, while among the 25

cases with endoscopic specimens, 13 (62.2%) had positive expression and the difference of CDX2 expression was statistically significant ($p < 0.001$) which is similarity to, Baker et al.,³⁶ and Shao et al.³⁷ With lower cases expression of CDX2 in biopsied specimen. In this study, the median CDX2 score of malignant cases was significantly lower than precancerous lesions ($p = 0.019$). In the present study, among the 42 endoscopic biopsy samples, no significant association was observed between CDX2 expression and type of lesion ($p = 0.999$). As stated by Lorant et al.,³⁹ endoscopic biopsy samples are adequate quality to enable reliable classification of gastric cancer, about differentiation, the presence of signet ring cells, histologic types and a difference with premalignant lesion, such as dysplasia. There are no major differences between the histological types of lesions in biopsy samples. Moreover, this study revealed that, among the 60 cases with malignant tumor, 37 (61.7%) cases had positive CDX2 expression while among the 17 cases with precancerous lesion, 15 (88.2%) had positive CDX2 expression. CDX2 expression is significantly high in precancerous cases than malignant cases ($p = 0.039$) which has resemblances with studies by Qin et al.,³¹ and Xiao et al.⁴⁰ According to Lauren classification, the present study revealed that among the 60 malignant cases, intestinal type gastric cancer expressed significantly high positive CDX2 expression (76%) than diffuse (23.1%) and indeterminate (40%) type GC cases ($p = 0.001$). This is similar to the study by Akbari et al.,²¹ Halder et al.,²⁵ and Qin et al.,³¹ with higher CDX2 expression among intestinal type GC. However, Wang et al.¹⁷ and Qin et al.,³¹ stated significantly high CDX2 expression in well and moderately differentiated GC ($p < 0.00001$ and $p = 0.01$). In this study, among the 60 malignant cases, two-tiered grading system of WHO 2019 classification was applied, which revealed 40 cases were of low-grade types, of which 31 cases (77.5%) had positive CDX2 expression. This study demonstrated that, significantly high CDX2 expression was associated with low grade gastric cancer ($p < 0.001$) which is similar to studies by Saito et al.,¹⁵ and Wang et al.¹⁷ In this study, CDX2 expression was positive in majority ($n = 31$, 77.5%) of tubular adenocarcinoma cases than other GC types. However, CDX2 expression tends to be heterogeneous as compared with strong staining with intestinal type tubular adenocarcinoma.⁴¹ According to this study, there was no statistically significant association between CDX2 expression and pT ($p = 0.321$) and pN ($p = 0.156$) staging, which is similar to Qin et al.,³¹ and Park et al.⁴² In contrast Camilo et al.,⁴³ observed inverse correlation between CDX2 expression and pTNM stage ($p = 0.002$). In this study, no significant association was observed between CDX2 expression and grading of intestinal type gastric carcinoma ($p = 0.424$) which is contrast to Helal et al.⁴⁴ They observed increased CDX2 expression associated with low grade intestinal type GC cases ($p > 0.001$). The inconsistency between the results might be due to relatively small sample size in this study.

CONCLUSION

In conclusion, CDX2 could be a good prognostic marker and helpful in differentiating different histological types and grades among GC cases. Positive expression was related to well and moderately differentiated, low grade, intestinal type and tubular adenocarcinoma cases. Precursor lesions presented with significantly high CDX2 expression than GC cases indicating a definitive tumor suppressive role of this marker in carcinogenesis. Biopsy specimens of malignant cases showed significantly increased expression and could be used as a diagnostic tool in this regard.

Limitations

1. Sample size was small.
2. The study population was selected from Dhaka city, so the results may not reflect the exact scenario of the country.

Recommendations

1. Large-scale studies are recommended in future for more conclusive result.
2. CDX2 immunostaining is recommended in preoperative endoscopic biopsy specimens.
3. Evaluation of the association between CDX2 expression and survival of patients is recommended.

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